THE DEVELOPMENTAL HISTORY OF AMAROECIUM CONSTELLATUM. II. ORGANOGENESIS OF THE LARVAL ACTION SYSTEM

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Introduction

The early development of the embryo of *Amaroecium constellatum* has been presented in a previous paper (Scott, 1945). The accumulation of yolk modifies the pattern of mosaic development characteristic of Tunicates to the extent that gastrulation is accomplished in an atypical manner. Convergence of the cells of the lateral margins of the posterior blastoporal lip is accomplished to the left of the mid-line. The neural plate elongates posteriorly at the place where the lateral blastoporal lips meet and close. The chordal cells are inflected at the anterior lip and lie in the median axis. The potential muscle cells of the morphological right side lie dorsal to the notochord as a result of their growth across the mid-dorsal plane, the muscle cells of the morphological left side lie below the level of the notochord on the curved left side of the embryo. The two groups of muscle cells are separated by the posterior extension of the neural plate.

Materials and Methods

Amaroecium constellatum is abundant along the eastern coast of the United States. The breeding season lasts throughout the summer months. Material for this study was collected at Woods Hole, Massachusetts. The embryos, squeezed from adult colonies, were selected and arranged into a progressive series of stages for study. They were fixed in Bouin's fluid. Some were stained by Conklin's modification of Delafield's haematoxylin, others with borax-carmine, then cleared according to the benzyl-benzoate method described in Romeis' "Taschenbuch der Mikroscopischen Technik." Corresponding stages were sectioned serially, stained in Mayer's or Gallagher's or iron haematoxylin, and counterstained with eosin or triosin. All drawings were made with the aid of a camera lucida. The photomicrographs were made with a Leitz "Macca" camera using Zeiss apochromat, 20 ×, and fluorite oil immersion, 100 ×, objectives with a Zeiss microscope.

Later embryonic development

It seems advisable to present a descriptive series of developmental stages that may be used as points of reference for structures differentiating during the organ-forming period. For convenience the developmental period following gastrulation is divided into four stages; 1) the tail bud stage, 2) early tadpole stage, 3) prehatching stage, and 4) the free-swimming tadpole stage. The free-swimming larva or tadpole has been described thoroughly by Grave (1921) and shall be presented here in brief summary since reference to it is necessary. A short description of the external appearance of these stages will be given first and referred to in subsequent treatment of organogenesis as Stages I, II, III, and IV. The terms, larval action system and adult action system, used by Grave (1935, 1944) will be adopted for the structures functioning during larval life and those functioning during adult life respectively.

The tail bud stage

By the end of gastrulation the embryo is approximately spherical except for a shallow postero-ventral invagination of the ectoderm constricting tail from trunk region. The furrow appearing first on the right side is deeper there, and less deep as it extends to the left side. The tail bud is short and rounded, curving immediately toward the ventral side of the trunk. Through the thin epidermis quadruple rows of large muscle cells can be seen lying dorsal and ventral to the notochord. The neural plate is elevated at the periphery to form the neural groove, enclosing anteriorly a wide depression, the presumptive brain region, posteriorly a narrow, trough-like depression lying to the left of the notochord, the presumptive neural tube (Fig. 1*A*).

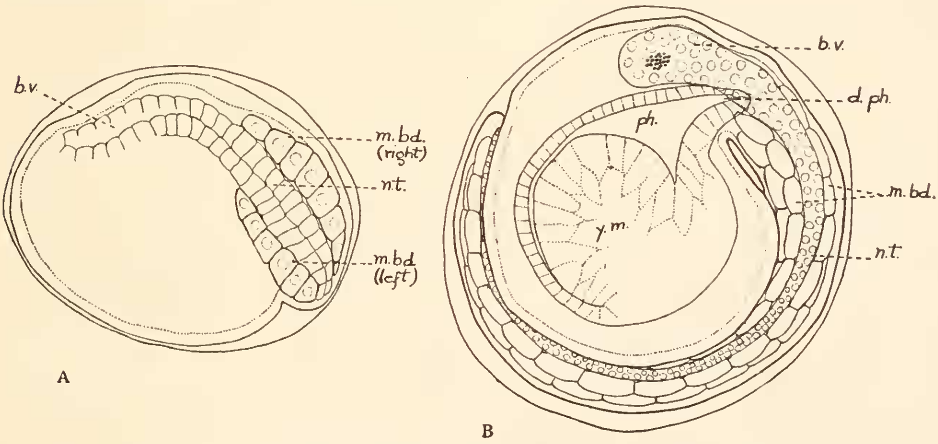

FIGURE 1. *A*. Stage I, embryo before neural folds close. 160 ×. *B*. Stage II, early tadpole; beginning of differentiation of digestive and nervous systems. 160 ×. *b. v.*, brain vesicle; *d. ph.*, dorsal diverticulum of pharynx; *m. bd.*, muscle band; *n. t.*, neural tube; *ph.*, pharynx; *y. m.*, yolk mass.

A transverse section through the tail bud stage discloses that the embryo is solid. A single layer of definitive endoderm lies under the concave neural plate (Fig. 5*E*). This layer of cells develops from the cells that form the superficial "pseudo-invagination" cavity of gastrulation. The depression closes by a reversal in change of shape of the cells involved rather than by approximation of the lips of the blastopore thus producing a solid archenteron (Scott, 1945). The endodermal cells spread under and anterior to the neural plate. Ventral to them is located the mass of heavily yolk-laden cells derived from the macromeres. Wedged between the thin ectoderm and the solid endoderm on either side is a mass of mesenchyme, small, polygonal cells with prominent nuclei (Fig. 5*E*).

Posteriorly the definitive endoderm lies adjacent to the chordal cells which are beginning to interdigitate in the base of the tail bud. The mesenchyme terminates abruptly in this region against the muscle cells of the tail.

Early tadpole stage

The embryo increases in size and acquires the shape that justifies its being called "tadpole." The trunk region elongates slightly in the antero-posterior axis remaining curved at the anterior end. The tail encircles the body meridionally as it grows in length. The embryo is still opaque.

The neural folds are closed, the position of the sensory vesicle being marked by aggregations of black pigment which show through the surface of the body. The neural tube is faintly visible along the side of the tail. More conspicuous are the large muscle cells dorsal and ventral to the prominent notochord which forms the axis of the tail throughout its length. Dorsally, on either side of the sensory vesicle there is a slight ectodermal invagination, rudiments of the atrial chambers. The embryo is confined within a test the cells of which are arranged in a compact layer (Fig. 1*B*).

Pre-hatching stage

Changes in the external appearance of the later embryo depend on the development of siphons and adhesive papillae and the secretion of a tunic. As body growth continues and organs of the larval action system differentiate, the body becomes transparent except where the mass of yolk is lodged in the pharynx.

The trunk region continues to elongate antero-posteriorly becoming elliptical in shape. Posteriorly the body narrows to the base of the tail; anteriorly it flares in the dorso-ventral axis in relation to the vertical position of the adhesive papillae. Laterally the body is compressed. A thickening layer of tunic invests the entire trunk. It is indented at the junction of trunk and tail and continues over the surface of the tail. The tail encircles the body meridionally being pressed into a groove in the tunic. The tunic of the tail projects laterally into fins.

The sensory vesicle occupies a dorsal position at the posterior end of the trunk. Two masses of pigment project into its cavity. Immediately in front of it lies the elevation of the oral siphon; behind it and on the posterior curve of the body lies the atrial siphon. Much of the internal structure is visible through the tunic and mantle. The incipient adhesive papillae appear as three disc-like projections in verticle series at the rounded anterior end (Fig. 2).

The free-swimming tadpole stage

The trunk of the tadpole of Amaroecium at its release measures about 600 micra in length; it measures about 270 micra in depth. The tubular atria with their triple rows of gill slits are pressed into the dorsal pharynx through half of its length posteriorly. An obvious structure in the pharynx is the dorsal, heavily ridged endostyle which seems to rest on the lateral masses of yolk that form the wall of the pharynx. The transparent pericardium occupies a large space below the yolk anteriorly in front of the loop of alimentary tract. On the right side of the body the stomach extends along the posterior and ventral curvature of the yolk. On the left the narrow intestine curves along the side of the stomach up to the left atrium where it terminates. The root of the tail lies in the posterior third of the length of the body.

Anteriorly, the adhesive papillae project into the tunic in a vertical row slightly to the right of the median plane. The test vesicles lie loosely within the tunic or many of them, even at the time of hatching, retain a slender connection with the cone or ridge from which they originate. Where the tail is continuous with the trunk the tunic dips down into an abrupt pocket. The epidermis secretes a thin sheath of tunic about the tail. Laterally it expands into wide sail-like fins (Fig. 3).

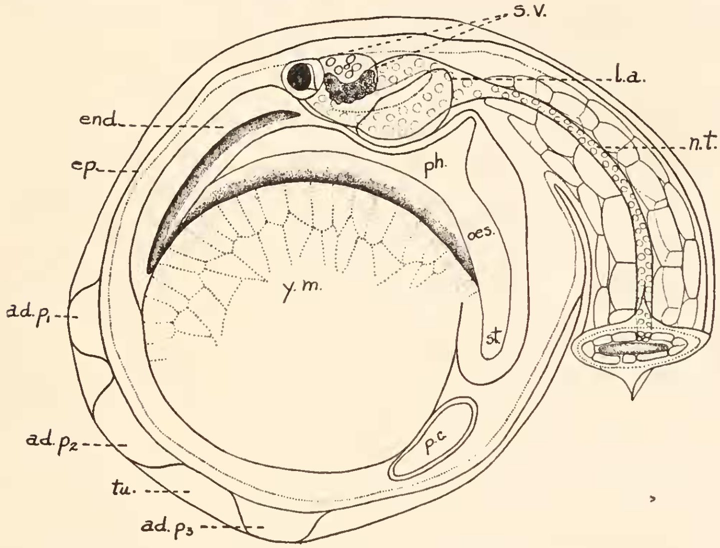

FIGURE 2. Stage III, lateral view of tadpole with incipient adhesive papillae. About 120 ×. *ad. p.*, adhesive papillae; *end.*, endostyle; *ep.*, epidermis; *l. a.*, left atrium; *n. t.*, neural tube; *oes.*, oesophagus; *p. c.*, pericardial cavity; *ph.*, pharynx; *st.*, stomach-intestine rudiment; *s. v.*, sensory vesicle; *tu.*, tunic; *y. m.*, yolk mass.

Organogenesis of the Larval Action System

Digestive system

The pharyngeal cavity develops in Stage II by delamination between the layer of definitive endoderm and the mass of yolk-laden cells, appearing first below the brain and spreading from that point (Fig. 1*B*, 5*F*, 6*E*). It extends back to the base of the notochord as an upwardly directed diverticulum. Ventral to the base of this projection a second invagination appears, the rudiment of the stomach and intestine located a little to the right of the median plane on the inner side of the visceral ganglion (Fig. 2, 6*F*).

The pharynx deepens in Stage III encroaching upon the mass of yolk cells. Gradually thin septa of epithelium divide the yolk mass into four compact longitudinal columns, the two on each side being continuous at the bottom. The central two are lower than the outer two, thus providing greater depth for the limited pharyngeal cavity (Fig. 6*A, F*). This supply of nutritive material in the pharynx remains to be digested during the active life of the larva and throughout the critical period of metamorphosis. All other tissues lose their meager supply of yolk almost entirely, leaving their cytoplasm clear.

Along the roof of the pharynx, anterior to the place of origin of the oral siphon, the epithelium rises up into a double fold enclosing the endostyle, restricted to the dorsal side above and between the lateral masses of yolk and passing to the anterior end of the yolk mass (Fig. 2, 6*A*). Before the tadpole is released from its test, the cells in the floor of the groove develop long cilia. The pharynx grows out above and below the atrial sacs, bringing the mesial atrial and lateral pharyngeal walls into intimate contact (Fig. 6*B*).

Due to the combined activity of atrial and pharyngeal epithelia, three horizontal rows of gill slits are formed, each consisting of seven or eight perforations. The

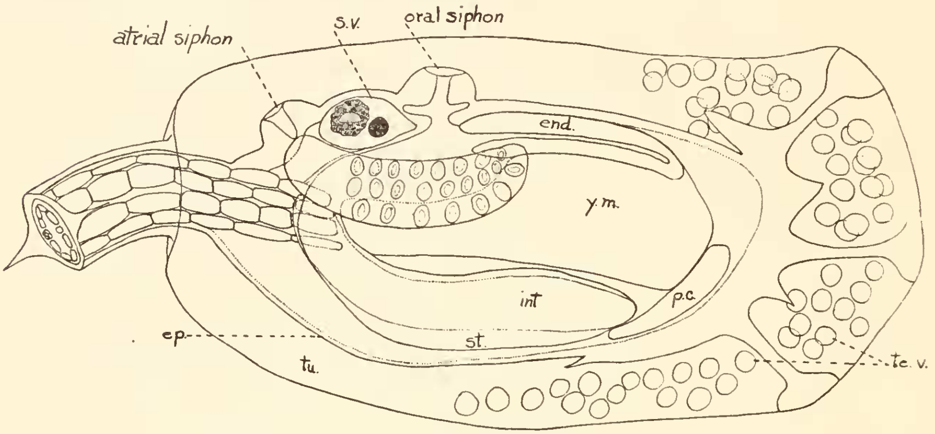

FIGURE 3. Stage IV, tadpole at hatching. About 120 ×. *end.*, endostyle; *ep.*, epidermis; *int.*, intestine; *p. c.*, pericardial cavity; *st.*, stomach; *s. v.*, sensory vesicle; *te. v.*, test vesicles; *tu.*, tunic; *y. m.*, yolk mass.

bordering cells of each gill develop a heavy brush of cilia, precocious equipment from the functional point of view. Even though the mouth breaks through to the branchial chamber, the tunic fills up the oral and atrial siphons until metamorphosis is completed.

The rudiment of the oesophagus grows forward along the curvature of the yolk and dilates to form the stomach. The diverticulum extends to the midventral region of yolk where it turns sharply upon itself and continues backward as the slender intestine. With a gradual slope upward the intestine retraces the course of the stomach on its left side terminating ventral to the posterior end of the left atrium (Fig. 3, 6*B*). Later the anus opens into the atrium here.

There are no cilia evident in the intestine or stomach during this period of development. The wall of the stomach is thicker than the wall of the remaining parts of the digestive tract although the alimentary epithelium, throughout its length, consists of a single layer of cells.

With rapid general growth of the body, the loop of intestine and stomach increases in length anteriorly, extending through the posterior half of the body cavity below and behind the yolk-laden pharynx (Fig. 3). The pericardium lies directly in front of it. Between the arms of the loop posteriorly are lodged the bases of the axial organs of the tail.

Atrium—During Stage II the atrium or peribranchial sac appears as a pair of ectodermal invaginations, one on either side of the sensory vesicle (Fig. *6E*). At the place of its origin the neck of each depression constricts and separates from the surface.

In the transition from Stage II to Stage III, the atria, in contact with the lateral endodermal wall of the pharynx, grow in an anterior direction only, with the result that the atrial chambers are horizontal capsular cavities located dorsally, one on either side of the pharynx (Fig. 6B). They extend through the posterior two-thirds of the trunk, curving gently upward posteriorly where they grow towards each other and unite behind the pharynx (Fig. 3). The atrial siphon opens through the dorsal wall of this connecting canal between the two cavities.

The atrial walls are characteristically thin and the cells lose their intercell membranes. Occasional yolk granules are scattered through the cytoplasm. During Stage III the gill slits perforate the walls in three horizontal rows on the inner side in direct contact with the wall of the pharynx. The lowermost row develops first, the atrial and pharyngeal fusing first in these regions. The slits number between seven and nine in each row. Later in the free-swimming period the cells bordering the gill aperture produce long cilia. The endoderm has no part in atrial formation except insofar as the gill slits are the product of joint activity of atrial and pharyngeal walls (Caullery, 1895).

Oral and Atrial Siphons—Late in Stage III the dorsal ectoderm in front of the sensory vesicle thickens and invaginates, pushing the endoderm of the pharynx before it. The circle of epidermis around the invaginated area becomes elevated, giving the oral siphon a crater-like appearance (Fig. *6B*). The floor of the invagination thins out in a flat layer against the pharyngeal roof with which it is in contact. The lower part of the cavity projects outward from the center and produces a ring-shaped extension on the mouth opening. The oral cavity assumes the shape of a flask with a long neck and a flattened base (Fig. 3). Into this ectodermal cavity, or stomodaeum, the hypophysial duct opens, just before hatching of the tadpole. Although the oral plate breaks through late in the tadpole's development, the tunic fills up the stomodaeal portion and prevents the passage of both food and water during larval life.

The atrial siphon, like the oral, is formed by ectodermal invagination. The thickened mantle is elevated, raising the siphon above the level of the rest of the mantle in knob-like fashion (Fig. *6C*). The floor of the invagination fuses with the dorsal wall of the connecting arm of the atrium. The atrial siphon is situated on the downward curve of the dorsal surface just posterior to the sensory vesicle and anterior to the insertion of the tail (Fig. 3, *6G, H*). The epithelial lining of the oral and atrial siphons projects into each opening at several points forming small tentacles. The mesenchymatous muscles in the mantle in this region provide the contractile elements that control the apertures when the siphons begin to function.

Heart and pericardium

Towards the end of Stage III, the endodermal cells extend completely around the yolk mass as a definite epithelium. Mid-ventrally it evaginates into the body space and constricts off from the yolk epithelium. The bladder-like vesicle is the pericardium which invaginates mid-dorsally into an inner enclosed vesicle, the heart. The cells lose their inter-cell membranes and the nuclei bulge irregularly in both cardial and pericardial walls (Fig. 6*A*). The heart does not develop beyond this point at present, the circulatory system not functioning during larval life.

The nervous system

The neural folds of Stage I close in the early phase of Stage II thus forming the hollow nervous system typical of chordates except in one point, the curving of the neural tube through 90° to the left of the brain region. The anterior portion of the nervous system produces the sensory vesicle with its sensory organs, the hypophysis, definitive ganglion, and the so-called subneural gland. The intermediate part including the origin of curvature and a small contribution from the brain region gives rise to the visceral ganglion and the spinal enlargement, the posterior part becomes the neural tube.

The cavity of the brain region is slightly dilated and its wall uniformly thick. The neural tube consists, in section, of four cuboidal cells surrounding a small lumen (Fig. 4*C*). Cell membranes in both regions are distinct at this stage, the nuclei are large and contain heavily staining nucleoli. The cytoplasm is reticular in appearance and has occasional yolk granules.

During Stage III the brain vesicle differentiates into two structures, the sensory vesicle in the entire right side and the rudiment of the hypophysis on the left posterior side (Fig. 4*A*, 5*A*). The vesicle expands, its walls becoming thin; the rudiment of the hypophysis remains small with thick walls. This secondary cavity is separated completely from the sensory vesicle at the region of evagination but their walls remain attached throughout subsequent development (Fig. 5*B*, *C*).

The sensory vesicle—Two sensory structures develop in the sensory vesicle, the statolith and the eye. The left posterior wall of the vesicle thickens, the right wall expands dorsally and laterally; all the cells lose their inter-cell membranes. The left wall of the cavity remains thick and constitutes the sensory ganglion of the brain. One cell on the ventro-anterior wall projects into the cavity and large pigment granules are deposited in its cytoplasm; these coalesce to form the statolith (Fig. 4*B*, 5*B*). In Stage IV the statolith is a spherical mass of pigment confined within the cell membrane and attached to the ganglionic wall by a stout stalk, the remaining part of the cell (Fig. 4*D*, 5*C*, *D*).

A group of cells situated dorso-laterally at the left posterior limit of the vesicle initiates the development of the eye by the deposition of pigment granules of much smaller size than those that form the statolith. Absence of cell membranes makes it difficult to ascertain the number of cells that participate in this activity. The pigment is deposited in the shape of a cup, its concavity facing dorso-laterally and to the right within the vesicle. Three ganglionic cells which retain their membranes fill up the concavity in series. They secrete globules of liquid which increase in size both by the gradual addition of the secretion and by the fusion of globules. The globules of liquid form the so-called lens cell (Fig. 4*A*, *B*, *D*, 5*D*). The nuclei

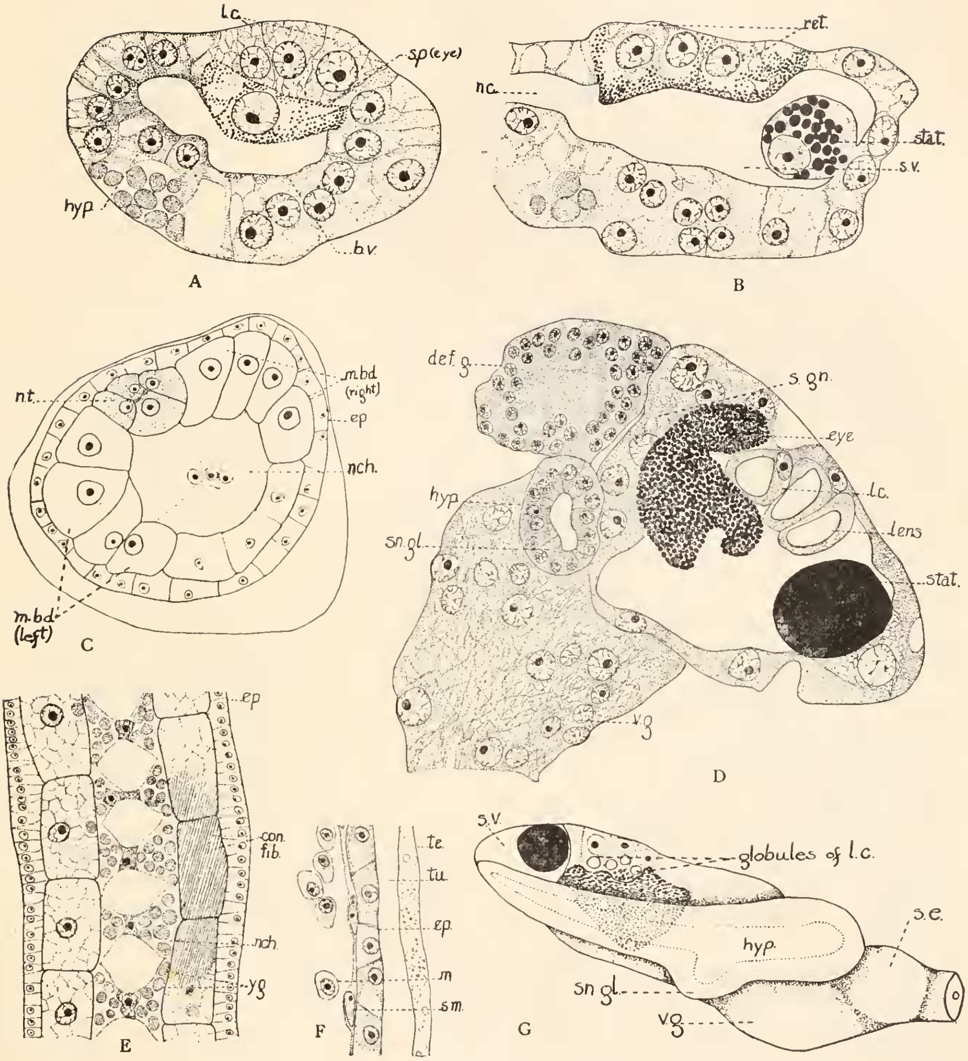

FIGURE 4. *A.* Transverse section through brain after the neural folds close, Stage II. 750 ×. *B.* Longitudinal section through brain of same stage. 750 ×. *C.* Cross section through tail of tadpole, Stage III. 300 ×. *D.* Section through brain of tadpole just before hatching, oblique to include both sensory organs. 750 ×. *E.* Longitudinal section through tail of tadpole in Stage III. 300 ×. *F.* Section through epidermis and test of Stage III. 750 ×. *G.* Reconstruction of brain and related structures of Stage III, viewed from left side. 300 ×. *b. v.*, brain vesicle; *con. fib.*, contractile fibrils; *def. g.*, definitive ganglion; *ep.*, epidermis; *hyp.*, hypophysis; *l. c.*, lens cell; *m. bd.*, muscle band; *n. c.*, neural canal; *nch.*, notochord; *n. t.*, neural tube; *ret.*, retinal cells of eye; *s. gn.*, sensory ganglion; *sn. gl.*, subneural gland; *stat.*, statolith; *s. c.*, sensory cell; *s. e.*, spinal enlargement; *s. m.*, smooth muscle cells of mantle; *s. p.*, sensory pigment; *t. c.*, test cells; *tu.*, tunic; *v. g.*, visceral ganglion; *y. g.*, yolk granules.

which at first occupy a central position in the cells are pushed to the periphery as the lenses, increasing in size, come eventually to monopolize the entire cell.

The pigment granules of the eye always remain discrete, not coalescing as do those of the otolith. Extending through the concentrated pigment are small rods of clear cytoplasm. They run from the base of the cup back towards the ganglion. Seven or eight of them may be seen in embryos of Stage IV that are mounted in a mixture of benzyl-benzoate and oil of wintergreen.

The hypophysis—The rudiment of the hypophysis early in Stage III appears as an extension or small evagination of the brain cavity (Fig. 4*A*, 5*A*). The cells retain their membranes, their nuclei are smaller than those of the adjoining part of the brain. Histologically they present the appearance of epithelial tissue. Upon its separation from the primary cavity during Stage III it elongates antero-posteriorly along the left side of the sensory ganglion (Fig. 4*G*). In Stage IV it ends blindly at the posterior wall of the oral siphon. Later these walls fuse and the hypophysis communicates with the posterior region of the stomodaeum, extending along the side of the ganglion with a gentle slope upward as far as the atrial siphon where it terminates blindly. The floor of the duct, corresponding in position to the region of the eye, deepens abruptly (Fig. 4*D*, *G*). The ventral wall of the pocket becomes slightly thicker, the indentation with its thickened floor constituting the subneural gland. Hjort (1896) reviews the opposing views concerning this structure in the early works on Tunicates.

The definitive ganglion—By a proliferation of cells in the mid-region of its roof in Stage II the hypophysial duct produces an oval mass containing small nuclei similar to those in the hypophysial duct itself. The cell membranes disappear and the nuclei wander out toward the periphery where they collect in several rows with the granular cytoplasm concentrated in the center (Fig. 4*D*, 4*G*, 5*C*). This part of the nervous system, the definitive ganglion, persists through metamorphosis and together with the hypophysis gives rise to the permanent nervous system of the adult.

Visceral ganglion—The visceral ganglion originates in that part of the neural plate that curves toward the left in Stage I. The lumen is obliterated, the large nuclei migrate to the periphery leaving the medulla mass of interlacing fibrils and granules (Fig. 4*D*). The visceral ganglion lies posterior to and ventral to the sensory vesicle. Dorsally where it merges with the sensory ganglion, it exceeds the sensory vesicle in diameter but it gradually diminishes in diameter towards the base of the tail where it is continuous with the neural tube. At the junction there is a slight enlargement called the spinal enlargement (Fig. 4*G*). The neural tube retains its lumen. It runs through the length of the tail to the left of the notochord. In Stage IV a single nerve emerges from the visceral ganglion on its right side just below the hypophysis. It runs anteriorly and sends out branches to the smooth musculature of the mantle.

The Notochord

At the end of the gastrulation period the chordal cells lie under the posterior part of the neural plate. Anteriorly adjacent to them are endodermal cells; dorsally, the potential muscle cells of the right lateral margin of the blastopore; ventro-laterally, the potential muscle cells of the left lateral margin of the blastopore. Posteriorly the chordal cells extend into the rudiment of the tail.

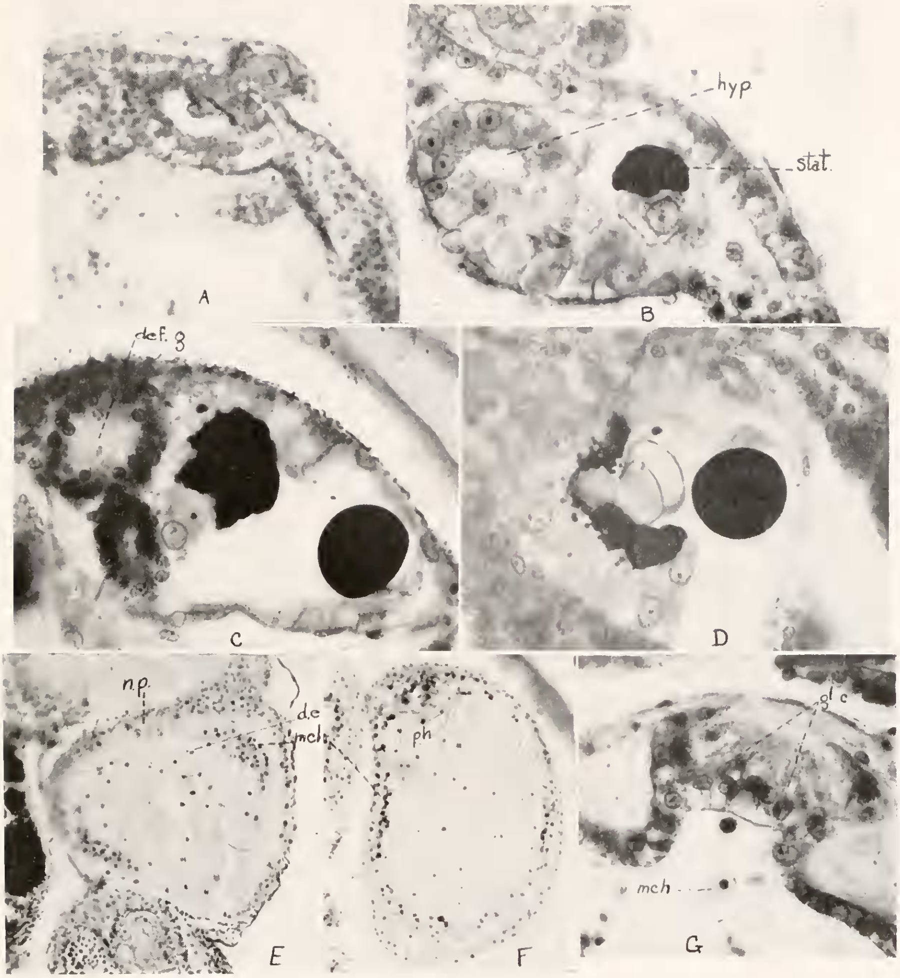

FIGURE 5. *A*. Transverse section corresponding to Figure 4*A*. 225 ×. *B*. Transverse section through brain of Stage III, hypophysis separated from brain vesicle. 650 ×. *C*, *D*. Sections through sensory vesicle and definitive ganglion of Stage IV; oblique, thus including both sensory organs. 650 ×. *E*. Transverse section through Stage I; anterior region. 150 ×. *F*. Transverse section through early Stage II; neural folds closed. 150 ×. *G*. Longitudinal section through adhesive papilla of Stage III. 650 ×. *d. e.*, definitive endoderm; *def. g.*, definitive ganglion; *gl. c.*, gland cells; *hyp.*, hypophysis; *mch.*, mesenchyme cells; *n. p.*, neural plate; *stat.*, statolith.

Some of the endodermal cells of the yolk mass lie along the right side of the chordal cells and when the tail is constricted from the trunk region these cells form the loose column of caudal endoderm. In Stage IV little of it remains (Fig. 4*C*, 6*B*).

In Stage I the notochordal cells begin to shift in position. They interdigitate into a row of disc-shaped cells occupying the central axis of the short tail. The cells resemble the endodermal cells of the yolk mass in possessing delicate membranes, nuclei smaller than those of adjoining muscle cells, and yolk granules.

During Stages II and III the notochord elongates as the tail lengthens. The chordal cells lengthen; the inter-cell membranes separate from each other converting them into hour-glass shaped cells with the nucleus resting in the constricted neck between the peripheral masses of protoplasm (Fig. 4*E*).

In Stage IV the cell halves separate completely giving the chord the appearance of a tube with a scalloped lining. The proximal end retains its relationship with the hinder end of the pear-shaped mass of yolk between the atrial cavities and the arms of the digestive tract (Fig. 6*C*, *H*). Distally it corresponds in length to the neural tube and tail muscles.

Muscle cells of the tail

Mesoderm differentiates into three structures of the larva, one of which is restricted to the larval action system, two of which function in both the larval and adult action systems. The former includes the muscles of the tail, the latter the muscles of the mantle and mesenchymatous connective tissue in the body cavity. The asymmetry of the posterior lip of the blastopore at the end of gastrulation (Stage I) places the muscle cells of the right side dorsal to the chordal cells and to the right of the neural plate at its posterior end, the muscle cells of the left side to the left of the posterior neural plate but ventral to the notochord (Fig. 4*C*). Each band is made up of four cells in fairly regular rows.

In Stage II the myoblasts are the most prominent cells in the body because of their size and heavy membranes. Each cell contains a large faintly reticular nucleus with a conspicuous nucleolus. The deeper cytoplasm is grossly reticular and retains an occasional yolk granule (Fig. 6*D*).

In Stage III the peripheral cytoplasm elaborates in its cortex, in a slightly spiral direction, along the longitudinal axis rows of contractile fibrillae composed of minute granules so distributed that they resemble the individual myofibrillae of striated muscle of the higher chordates (Fig. 4*E*, 6*D*). The myofibrillae are continuous from one cell to another throughout the length of the muscle bands. Grave (1921) has described this in the free-swimming tadpole of Amaroecium. The bases of the muscle bands, like that of the notochord in Stage IV, are located well within the posterior part of the trunk just behind the mass of yolk (Fig. 6*H*).

Muscles of the mantle

In the late embryonic period (Stage III) many of the mesenchyme cells located directly under the ectoderm unite end to end to form the smooth fibres of the mantle (Fig. 4*F*). One set of such muscle fibres radiates from each of the siphons. The other set encircles the trunk obliquely from the dorsal to the ventral side.

Mesenchyme of the body cavity

In Stage I two compact lateral masses of mesenchyme cells lie pressed tightly between the nutritive endoderm and shallow ectodermal cells. The one on the right side is disposed more dorsally than the one on the left side (Fig. 5*E*). They extend from the posterior muscle cells towards the anterior end of the body.

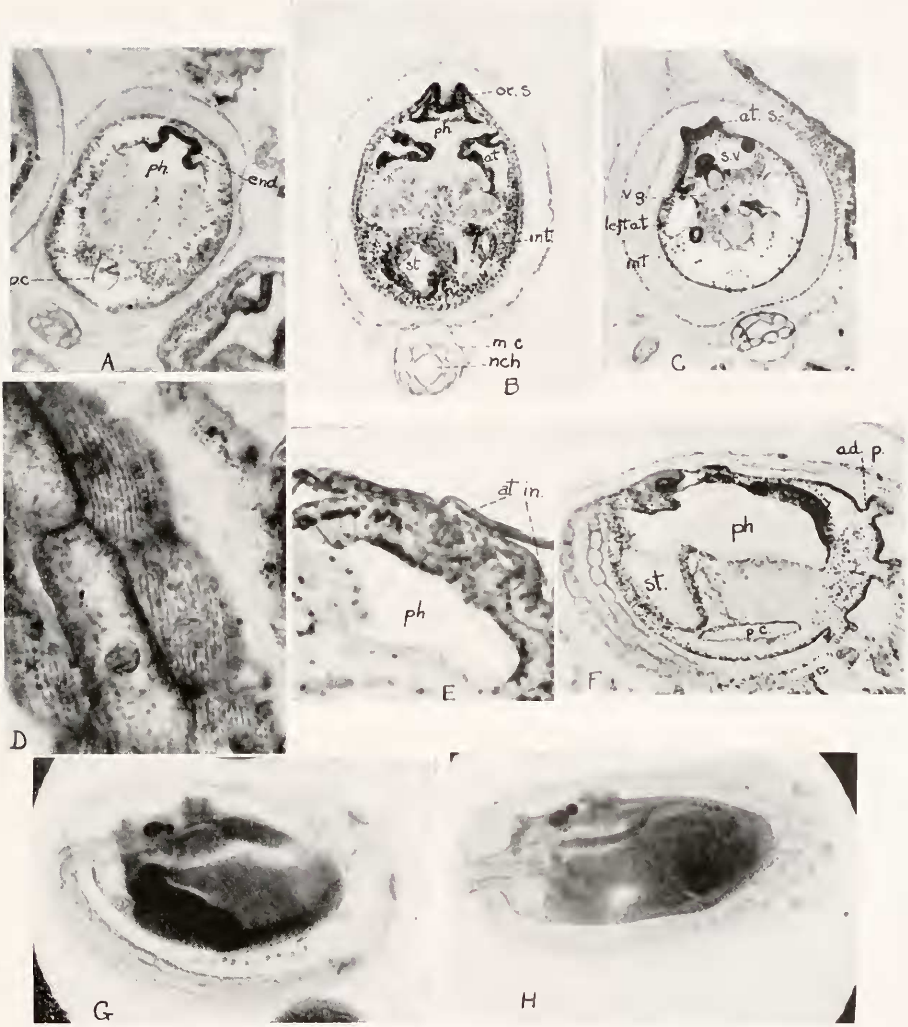

FIGURE 6. *A, B, C.* Transverse sections through tadpoles of Stage IV; *A*, anterior. *B.* In region of oral siphon. *C.* In region of atrial siphon. About 200 ×. *D.* Section through part of muscle band; middle cell through center of muscle cell, lateral cell through peripheral cytoplasm where myofibrillae are formed. About 850 ×. *E.* Transverse section through Stage II to show atrial invaginations. 300 ×. *F.* Longitudinal section through Stage III. About 150 ×. *G.* Tadpole just before hatching, chorion not ruptured. About 250 ×. *H.* Tadpole at hatching. Note insertion of notochord at posterior end of trunk. About 250 ×. *ad. p.*, adhesive papilla; *at.*, atrium; *at. in.*, atrial invagination; *at. s.*, atrial siphon; *end.*, endostyle; *int.*, intestine; *m. c.*, muscle cell; *nch.*, notochord; *or. s.*, oral siphon; *ph.*, pharynx; *p. c.*, pericardial cavity; *st.*, stomach; *s. v.*, sensory vesicle; *v. g.*, visceral ganglion.

In Stage II both masses of cells multiply and spread out under the ectoderm in all directions except posteriorly. A small amount of mesenchyme is found in the tail, probably derived from the cells in the mid-region of the posterior lip of the blastopore. Being crowded together the cells appear angular in section. The nuclei are relatively large (Fig. 4*F*, 5*G*).

During the transition from Stage II to Stage III growth of the body and absorption of the yolk effect a separation between the epithelial cells of the epidermis and the endodermal cells (Fig. 5*F*, 6*F*). As the body cavity enlarges the cells round up, separate from each other, and wander freely about, dividing frequently and eventually filling up all available space except in the area around the base of the tail (Fig. 6*A*, *B*, *C*).

Other mesenchyme cells assume stellate shape and send out long slender strands of protoplasm by means of which they form a reticulum of mesenchymatous tissue. This is the nearest approach to a coelomic epithelium that is found in Tunicates with the possible exception of the perivisceral cavity of Ciona.

The mantle and tunic

The epidermis in Stage I is a layer of thin cells small in surface view dorsally where they adjoin the neural plate, larger towards the ventral body region. In Stage II the cells are of uniformly small size and cuboidal in section except where they invaginate to form the atrium and are columnar in shape. In surface view all present the characteristic polygonal arrangement of epithelial tissue.

During Stage III the protoplasm becomes vacuolated medially, the nuclei being pushed to the periphery where the cytoplasm is more granular (Fig. 5*B*, 6*E*). The epidermal cells grow thinner as development progresses and the inter-cell membranes disappear. When the epidermis has assumed the characteristic appearance of the Tunicate mantle in Stage III it secretes a thick layer of structureless tunic. Occasional cells of the test of the ovum are trapped in the clear tunicin, the greater number, however, being pushed with the test ahead of the tunic (Fig. 6*C*). The tunic is grooved where it is secreted about the tail and when the tail is released, with the disappearance of the test, the groove remains in evidence marking the embryonic position of the tail.

Derivatives of the epidermis

Adhesive papillae—A conspicuous feature of the Amaroecium larva is a vertical row of three adhesive papillae at the anterior end (Fig. 3, 6*F*, *G*, *H*). Each papilla first appears early in Stage III as a local thickening of ectoderm forming a pad of columnar cells. The cells at the periphery of the thickened pad form a stem which increases in length as the tunic thickens, the whole organ becoming goblet-shaped. It retains its connection with the body cavity through its slender hollow stem (Fig. 6*G*, *H*). The papillae extend through the thickness of the tunic and are exposed at its surface. Cells that constitute the functional portion become vacuolated and reticular proximally and toward the center of the cup, where the long cells converge, they produce secretion granules which lodge in the concavity of the papilla (Fig. 5*G*). The bordering epidermis surrounds the disc forming a thin layer over the cup-shaped depression. During the free-swimming life of the larva the secretion granules are converted into a viscid substance by means of which the tadpole becomes attached. The entire glandular structure is of ectodermal origin. Grave (1921) from his study of the fully formed tadpole supposed that mesenchyme cells gave rise to the glandular portion of the papilla. Mesenchyme cells wander from the body cavity into the hollow stalk but they are not incorporated into its structure. The tail encircling the body crowds the papillae a little to the right of the sagittal plane thus adding to the asymmetry of the larva. The three papillae cannot be homologized with the tactile papillae of Botryllus, which are integral parts of the peripheral nervous system. Here they serve only as gross organs of attachment.

Test vesicles—During Stage III, when the adhesive papillae are differentiating, the test vesicles originate as numerous small ectodermal evaginations in four distinct regions at the anterior end of the trunk. Two groups, separated from each other by the median papilla, are directed forward. The dorsal group is derived from a short ridge extending in the direction of the oral siphon. The ventral group, below the ventral papilla, is derived from a long ridge extending posteriorly through about a third of the length of the trunk (Fig. 5, *6G*, *H*). The vesicles themselves originate as independent hollow slender projections of the ectoderm. The attached end of each evagination becomes narrow, finally constricting off and severing its connection at the base. Frequently this separation is not effected by the time of hatching of the vesicle still being attached to the epidermis by their stalk-like bases. When detached the slightly pear-shaped vesicle rounds up and becomes a sphere consisting of a single layer of cells which lose their definition on the proximal side where they are extremely thin.

The use of the word "test" in connection with these vesicles is unfortunate. The chorion of the egg of Tunicates is called the test and the cells that either lie freely in the enclosed liquid or are resolved into pavement epithelium are called the test cells. The tunic of the tadpole is a purely ectodermal derivative. The tunic of the adult colonies being the product of secretory activity of these vesicles, the vesicles should, with greater accuracy, be called the "tunic vesicles."

Summary

1. The digestive system of Amaroecium lacks an open archenteron at the end of gastrulation. The pharynx appears as a narrow incision with a thin roof and heavy floor. An oesophageal evagination differentiates into stomach and intestine.

2. Heart and pericardium originate from the floor of the pharynx.

3. Atrium and siphons are ectodermal structures that become associated with the digestive system.

4. The nervous system consists of a sensory vesicle enclosing two sensory masses of pigment, a hypophysis lying beside two sensory ganglion, a visceral ganglion descending laterally to the neural tube which lies to the left of the notochord throughout the length of the tail.

5. The notocord is derived from chordal cells invaginated at gastrulation. Its cells become vacuolated. The notochord is confined to the tail and posteriormost region of the trunk.

6. Muscle cells differentiate from mesodermal cells of the blastoporal margins. Asymmetry of the blastopore places the cells of its right margin dorsal to the noto-

chord, the cells of the left margin ventral to the notochord. Each band of muscle cells consists of four longitudinal rows. Cells separate from the two lateral masses of mesenchyme and move into the body space of the developing tadpole. They give rise to muscles of the mantle.

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